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NUTRITIONAL REQUIREMENTS IN CHOLESTEROL AND PHOSPHOLIPIDS FOR THE CHINESE PRAWN (*Penaeus Orientalis*)*

by ZHOU Hongqi and WANG Yiqiang
(Shanghai Fisheries University)

Abstract

A cholesterol diet test was designed using the gradient method, while the "two factors and three levels" were used in designing a cholesterol and phospholipid diet test. Findings from the tests show that the addition of cholesterol to the diet of the Chinese prawn can significantly promote the growth and improve the survival rate of the prawns. Cholesterol is an essential nutrient that has to be added to the diet of prawns. Judging from the growth and survival of the prawns being tested, as well as from the amount of cholesterol in their muscles, the addition of 0.5% cholesterol to the diet of prawns can satisfy their nutritional requirements.

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Phospholipids are also nutrients essential to the Chinese prawn. The addition of phospholipids to their diets can improve their survival rate and increase the cholesterol content of their muscles. In this experiment, when their diet contained 3% soybean oil, 2.5% fish oil and 0.5% cholesterol, a phospholipid content of 3% was able to satisfy the nutritional requirements of the prawns.

Key words

Chinese prawn (*Penaeus orientalis*), cholesterol, phospholipid

The Chinese prawn (*Penaeus orientalis*) is a major cultivated species in China. Rapid developments made in the prawn cultivation industry have given impetus to research on suitable formulated diets for the prawns. Since 1970, significant achievements have been made in research work on the nutritional requirements of the Chinese prawn. HOU Wenpu (1989) [1], XU Xinzhang *et al.*(1989) [2] reported on the optimum amount of protein, sugar and fat in the diet of prawns. LI Aijie *et al.* (1981-1985) [3] analyzed the amino acid requirements of the Chinese prawn at different stages of growth. RONG Changkuan *et al.*(1988) [4] conducted studies on the amino acid

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[1] HOU Wenpu 1989. "Guoneiwai duixia peier de yanzhi ji danbaiyuan de kaifa wenti [Research in China and abroad on the preparation of formulated diets for prawns and the question of finding new sources of protein]". *Quanguo duixia, yulei peihe erliao lunwenji [Collection of Essays on Formulated Diets for Prawns and Fish in China]*, 8-15.

[2] XU Xinzhang *et al.*, 1989. "Zhongguo duixia peier zhong danbaizhi, tang, xianweisu, zhifang de shiyi hanliang ji rixuyaoliang de yanjiu [A Study on Optimum Amounts and Daily Requirements of Protein, Sugar, Fibre and Fat in the Formulated Diets of Chinese Prawns]". *Quanguo duixia, yulei peihe erliao lunwenji [Collection of Essays on Formulated Diets for Prawns and Fish in China]*, 115-124.

(3) LI Aijie *et al.*, 1981-85. "Dongfang duixia anjisuan hanliang de fenxi [Analysis of the Amino Acid Content in *Penaeus orientalis*] ". *Duixia yingyang de yanjiu [Research on Prawn Nutrition]*, 18-30.

requirement in the dietary protein of the Chinese prawn. LI Hefang *et al.*(1987) analyzed the amounts of inorganic elements in the bodies of the Chinese prawn [1-5]. LI Aijie *et al.* (1986) studied the need for calcium and phosphorus in the diet of prawns. However, no report has yet been seen on the nutritional requirements of Chinese prawns for cholesterol and phospholipids.

Cholesterol and phospholipids are essential nutrients in the diets of the Chinese prawn. Teshima *et al.*(1971) pointed out that Japanese prawns are not themselves capable of synthesizing acetic acid into cholesterol. Teshima *et al.*(1982), Shudo *et al.*(1971) and Deshimaru *et al.*(1984) pointed out that juvenile Japanese prawns must rely on the cholesterol in their diet to maintain normal growth and survival. Castell *et al.*(1975) drew the same conclusion from studies on juvenile American lobsters. Teshima *et al.* (1982, 1986) conducted studies on the nutritional requirement of juvenile Japanese prawns for phospholipids and cholesterol. The objective of this present experiment is to discover the nutritional requirement of mature Chinese prawns for cholesterol and phospholipids in their feed diets, thereby providing a theoretical basis for formulating a standard for the amount of cholesterol and phospholipids in the formula feed diet of prawns.

Materials and Methods

A test was conducted in the Feng Xian County Prawn Nursery, Shanghai, between August 4 and September 11, 1987. The prawns used in the test were kept in a cement pond in the overwintering room. Before the test, the pond was sterilized with formaldehyde. The density of the pond was maintained at about 10 prawns per m^2. Oxygen was pumped into the pond water 24 hours a day. The water was changed at 2-3 p.m. daily, and about 4/5 of the total amount of water was changed. Another test was also conducted at the Shui

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(4) RONG Changkuan *et al.*, 1988. "Guanyu duixia siliao danbai zhong shizhong bixu anjisuan shiyi zucheng bili de chubu shiyan yanjiu [Preliminary Experimental Studies on Optimum Ratios for 10 Kinds of Essential Amino Acids in the Dietary Protein of Prawns]" I, II. *Quanguo xumu shuichan kaifa liyong keji jiaoliuhui lunwenji [Collection of Papers Presented at the China National Exchange Conference on Technologies in Husbandry and Fishery Development and Application]*, 57-70.

Dang Prawn Breeding Farm, Hai Yan, Zhejiang Province, between September 14 and October 7, 1988. The prawns used in the test were kept in a covered mesh box (measuring 100 x 80 x 60 cm^3). The box was placed in an overflow pond and cleaned every other day to ensure the unimpeded flow of the water inside and outside the box. During the test, the water temperature was maintained at 20 - 31.9°C, and the salinity at 4.7 - 7.0%. The pH level was about 8. The amount of dissolved oxygen was more than 3.5 mg per litre, and the amount of ammonia nitrogen was less than 0.7 mg per litre.

In 1987, the gradient method was used to design the dietary test. The concentration of cholesterol in the diet was set at 0%, 0.2%, 0.5% and 1.0% respectively. The basic composition of the diet was: egg albumen, 54%; starch, 20.5%; fish oil, 2.5%; vitamin complex, 2.5%; soybean oil, 3%; compound inorganic salts,12%; and algin, 3%. Four kinds of diets were formulated for the test (Table 1). In 1988, the dietary test was designed using the "two factors and three levels". The amounts of cholesterol and phospholipids tested in the diet were factors A and B respectively. The cholesterol levels were 0.5%, 1.0% and 1.5% respectively. The phospholipid levels were 1%, 3% and 5% respectively. Except for 50.5% casein and 20% dextrin, the basic composition of the diet was similar to that in 1987. A total of nine diets were formulated (Table 2).

At the start of the test, to avoid injury and subsequent death to the prawns being tested as a result of excessive handling, the initial body weight of the prawns was determined by weighing about 30 prawns selected from the same pond, whose sizes were similar to those of the test prawns. Alternatively, the average weight was obtained by dividing the total weight of the test prawns by their number.

The amount of cholesterol in the muscles of the test prawns was determined as follows: the samples were extracted using chloroform; after saponification, they were placed in petroleum ether, the extracting solution, and blow-dried in a water bath at 70°C, then dissolved in glacial acetic acid; ferric ammonium sulphate was added for coloration, and a colorimetric determination was carried out.

Findings

In 1987, prawns weighing an average of 7.0 - 8.2 gm were tested for 39 days. The findings, as seen in Table 3, show that the group of prawns feeding on diets without cholesterol scored significantly lower in terms of both weight gain and survival rate when compared with groups that fed on diets with varying amounts of cholesterol. This proves that cholesterol can promote the growth of prawns and improve their survival rate. Cholesterol is an essential nutrient in the diet of prawns. The optimum level of cholesterol added to their diets is 0.5%. At this level, the survival rate is as high as 85.5% and the rate of weight gain is 35.1%. A diet with 1% cholesterol achieves the second best effect.

In 1988, prawns with an average body weight of 11.4 gm underwent 24 days' testing with regard to their nutritional requirement for cholesterol and phospholipids. Each group consisted of 20 prawns. The figure attached to Table 4 shows the survival rate of the prawns being tested. From the representational data, it can be seen that the optimum survival rate was reached when the phospholipid level was at 3%. Group 8 (A_3B_2) came first, group 2 (A_1B_2) came second, while group 5 (A_2B_2) came third. Analysis of variance (Table 5) shows that the phospholipid content of the diet has significant bearing on the survival rate of the prawns ($p < 0.01$). By using the LSR method to compare the average survival rates obtained from different levels of phospholipids added, it was found that there was a significant difference between 3% and 1%, a highly significant difference between 3% and 5%, but no significant difference between 1% and 5%. The statistical findings match the data in representational form. The addition of 3% phospholipids to the diet can result in an optimum survival rate for the prawns, which is significantly different from that obtained from diets with different levels of phospholipids. Analysis of variance also shows that an increase from 0.5% to 1.5% in the cholesterol content of the diet cannot improve the survival rate of the prawns. Hence, there is no significant difference between groups 2, 5 and 8. For the survival of the prawns, 0.5% cholesterol in their diet is sufficient to satisfy their nutritional requirement. The best group should be group 2.

The effect of the cholesterol and phospholipid content of the diet on the growth of prawns can be seen in Table 6. As the refined diets used in the test did not quite appeal to the prawns' appetites, the prawns ate very little, and as a result, the growth rate in all

groups was rather slow. From the representational data, it can be seen that group 2 has the best growth rate. However, analysis of variance for the rates of weight increase in the prawns shows that the level of cholesterol and phospholipids in their diets has no significant effect on this rate ($p>0.1$). In other words, the growth of the prawns cannot be promoted even by increasing the cholesterol content of their diets from 0.5% to 1.5% and the phospholipid content from 1% to 5%. Therefore, looking at the question from the growth of the prawns alone, 0.5% cholesterol and 1% phospholipids can already satisfy the nutritional requirement of the prawns.

Table 7 shows the amount of cholesterol in the muscles of the prawns tested in 1988. Analysis of variance shows that the phospholipid level of the diets has a significant bearing on the cholesterol content of the prawns' muscles ($p<0.05$). When the phospholipid content of the diet was increased from 1% to 5%, there was a tendency for the amount of cholesterol in the prawns' muscles to increase. However, when the cholesterol level of the diet was maintained at 0.5% - 1.5%, the level of cholesterol had no significant effect on the cholesterol content of the muscles.

Discussion

Teshima and Kanazawa (1971) used the radioactive tagging method to demonstrate the inability of mature Japanese prawns to biosynthesize cholesterol. Whitney (1969) pointed out that juvenile crabs (*Rhithrapanopeus harrissi* and *Libinia emarginate*) are also incapable of doing so. Tests on the diets of Japanese larval and juvenile prawns show that the addition of cholesterol to their diets can effectively promote the growth, metamorphosis and survival of prawns (Kanazawa *et al.*, 1971; Shudo *et al.*, 1971; Deshimaru *et al.*, 1974; Teshima *et al.*, 1982, 1986). A similar conclusion was reached in a study involving larval American lobsters (Castell *et al.*, 1975). Teshima *et al.* (1986) analyzed the composition of prawns' bodies in a test on cholesterol feeds and succeeded in demonstrating the importance of cholesterol in the feed diet. This present test takes a look at the rate of weight increase and survival rate of mature Chinese prawns and finds a significant difference between the group that feeds on a diet without cholesterol and the group that feeds on a diet with cholesterol added. This proves that the addition of cholesterol to the diet is of utmost importance to Chinese prawns as well. Therefore, we believe that prawns, whether they are in the larval, juvenile or mature stage, require steroid substances from

external sources. Cholesterol is the main kind of sterol in prawns, making up approximately 90%. It is the precursor in the synthesis of vitamin D as well as of such steroid hormones as brain, moulting, and sex hormones. Hence, cholesterol is an essential nutrient for prawns.

A study on the optimum amount of cholesterol in the diet of mature Chinese prawns shows that for prawns weighing over 7.0 gm, the addition of 0.5% cholesterol in the diet is enough to ensure good growth and survival. Increasing the amount from 0.5% to 1.0% or even 1.5% cannot effect any improvement in the prawns' growth and survival. This shows that 0.5% cholesterol is sufficient to satisfy the prawns' nutritional requirement. On the other hand, when the cholesterol level in the diet exceeds 0.5%, it has no significant effect on the amount of cholesterol found in the prawns' muscles. This shows that an excessive amount of cholesterol added to the diet is of no use to the prawns and will only result in wastage. In considering how to satisfy the nutritional requirement of prawns for cholesterol to achieve the optimum physiological effect while also making the most effective use of cholesterol to cut down on the cost of diets, we determined that the optimum amount of cholesterol in the diet should be 0.5% for prawns over 7.0 grams. The nutritional requirement for cholesterol in juvenile American lobsters (Castell *et al.*, 1975) and juvenile Japanese prawns (Kanazawa *et al.*, 1971) is also 0.5%. However, Deshimaru *et al.* (1974) maintained that the nutritional requirement for cholesterol of juvenile Japanese prawns should be 2%. Shudo *et al.*(1971), on the other hand, felt that the optimum level should be maintained at 0.2%. Teshima *et al.* (1982) felt that the optimum level for larval Japanese prawns should be 1%.

Phospholipids are also a necessary nutrient in the diet of juvenile American lobsters (Conklin *et al.*, 1980; D'Abramo *et al.*, 1981) and juvenile Japanese prawns (Teshima *et al.*, 1986; Deshimaru *et al.*, 1982). The addition of phospholipids to the diet can promote the growth and survival of prawns. When the amount of phospholipids in the diet was increased from 1% to 3% in this test, the survival rate of the prawn improved correspondingly. However, when the amount of phospholipids was increased from 3% to 5%, their effect on the survival rate became less obvious. This is different from the effect of phospholipids on the survival rate of larval Japanese prawns (Teshima *et al.*, 1982), where the effect became more obvious as the level of phospholipids increased. The effect of phospholipids on the growth of prawns, as observed in this

test, was the same as that observed by Teshima *et al.* (1982). When the amount of phospholipids in the diet was within the range of 1% - 5%, the effect of phospholipids on growth was unrelated to the amount added. Therefore, when considering the simultaneous effect of phospholipids on survival rate and growth, the optimum level of phospholipids should be 3%.

Lester *et al.* (1975) were of the opinion that the nutritional value of phospholipids lay in their ability to help the digestion and absorption of cholesterol in the intestines. However, Teshima *et al.* (1983) felt that even if the diet did not contain phospholipids, the rate of digestion and absorption of cholesterol and triglycerides in the intestines could still reach 85%. Only when the prawns were fed on diets devoid of fats was the rate of digestion and absorption of cholesterol low. The radioactive tagging tests conducted by Teshima *et al.* (1986) showed that phospholipids could promote growth and improve the survival rate mainly because they are able to help in the transportation of fats, particularly cholesterol. The findings of our test also prove that phospholipids can help to transport cholesterol and keep them in the muscles. It can thus be seen that the nutritional value of phospholipids is related to dietary cholesterol and fat. In terms of satisfying the nutritional requirement of prawns, this experiment suggests that when a formula feed diet contains 3% soybean oil, 2.5% fish oil and 0.5% cholesterol, the optimum quantity of phospholipids is 3%.

表 1　对虾对胆固醇营养需要的试验设计(1987)

Table 1　Test design of nutritional requirement of cholesterone for the prawn(1987)

试验组[1] \ 成分含量(%)[2]	胆固醇[3]	磷　脂[4]	微晶纤维素[5]	基础饲料[6]
1	0	1	1.5	97.5
2	0.2	1	1.3	97.5
3	0.5	1	1.0	97.5
4	1.0	1	0.5	97.5

Key to Table 1:

1. Test group; 2. content (%); 3. cholesterol; 4. phospholipids; 5. microcrystalline cellulose; 6. basic diet.

表 2 对虾对胆固醇、磷脂营养需求的试验设计(1988)

Table 2 Test design of nutritional requirement of the prawn for cholesterone and phospholipid (1988)

试验组[1] \ 成分含量(%)[2]	(A)胆固醇[3]	(B)磷脂[4]	微晶纤维素[5]	基础饲料[6]
1(A_1B_1)	0.5	1	5	93.5
2(A_1B_2)	0.5	3	3	93.5
3(A_1B_3)	0.5	5	1	93.5
4(A_2B_1)	1.0	1	4.5	93.5
5(A_2B_2)	1.0	3	2.5	93.5
6(A_2A_3)	1.0	5	0.5	93.5
7(A_3B_1)	1.5	1	4	93.5
8(A_3B_2)	1.5	3	2	93.5
9(A_3B_3)	1.5	5	0	93.5

Key to Table 2:

1. Test group; 2. content (%); 3. cholesterol; 4. phospholipids; 5. microcrystalline cellulose; 6. basic diet.

表 3 饲料内胆固醇水平对于对虾生长及存活率的影响

Table 3 Effect of cholesterol levels in the diet on growth and survival of the prawn

胆固醇含量(%)[1]	试验开始[2]		试验结束[3]		存活率(%)[6]	增重率(%)[7]
	尾[4]数	平均体重[5](克)	尾[4]数	平均体重[5](克)		
0	128	8.2	33	9.1	25.8	11.0
0.2	120	7.0	57	9.1	47.5	30.0
0.5	131	7.4	112	10.0	85.5	35.1
1.0	114	7.5	91	9.8	79.8	30.1

Key to Table 3:

1. Cholesterol content(%); 2. beginning of test; 3. end of test; 4. number of prawns; 5. average body weight (gm); 6. survival rate (%); 7. rate of weight increase (%).

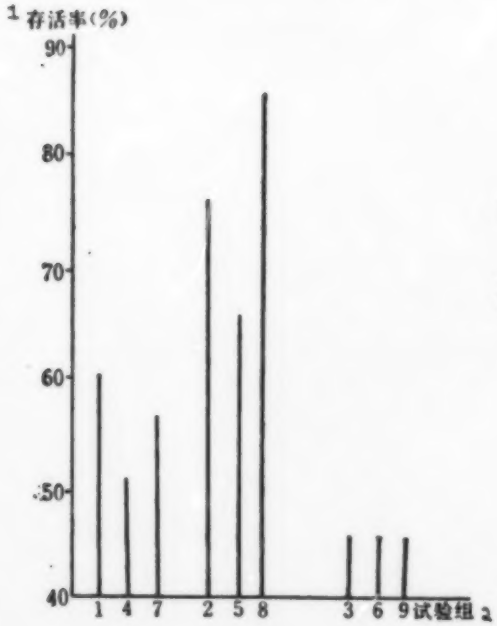
表 4　饲料内胆固醇、磷脂水平对于对虾存活率的影响

Table 4　Effect of cholesterol and phospholipid levels in the diet on survival of the prawn

1 磷脂含量 \ 胆固醇含量 2	存活 3 率 (%)			4 平均存活率(%)
	0.5(A_1)	1.0(A_2)	1.5(A_3)	
1(B_1)	60	50	55	55±7.07
3(B_2)	75	65	85	75±10
5(B_3)	45	45	45	45±0

Key to Table 4:

1. Phospholipid content; 2. cholesterol content; 3. survival rate (%); 4. average survival rate (%).



附 图　饲料内胆固醇、磷脂水平对于对虾存活率的影响

Attached fig. Effect of cholesterol and phospholipid levels in the diet on survival of the prawn

Key to attached figure:

1. Survival rate (%); 2. test group.

表 5 试验虾存活率的方差分析

Table 5 Analysis of variance on the data of the prawn survival

[1] 变异来源	[2] 自由度	[3] 平方和(SS)	[4] 方差(S^2)	F	$F_{0.05}$	$F_{0.01}$
[5] 胆固醇	2	116.67	58.34	1.75	6.94	18
[6] 磷 脂	2	1400	700	21(显著)	6.94	18
[7] 误 差	4	133.33	33.33			
[8] 总变异	8	1650				

Key to Table 5:

1. Source of variation; 2. degree of freedom; 3. squariance (SS); 4. variance (S^2); 5. cholesterol; 6. phospholipids; 7. error; 8. total variation.

表 6 饲料内胆固醇、磷脂水平对于对虾增重率的影响

Table 6 Effect of cholesterol and Phospholipid levels on body weight gain of the prawn

[1] 磷脂含量 \ [2] 胆固醇含量	[3] 增重率 (%)		
	0.5(A_1)	1.0(A_2)	1.5(A_3)
1(B_1)	28	28.9	29.8
3(B_2)	33.3	28	28.9
5(B_3)	28.9	25.4	28.7

Key to Table 6:

1. Phospholipid content; 2. cholesterol content; 3. rate of weight increase.

Table 7 Effect of cholesterol and phospholipid levels in the diet on content of cholesterone in muscle of the prawn

[1] 磷脂含量 (%) \ [2] 胆固醇含量 (%)	[3] 湿虾肉中胆固醇含量 (mg/g)		
	0.5	1.0	1.5
1	1.5	0.97	1.24
3	1.76	1.31	0.86
5	2.37	1.61	1.98

Key to Table 7:

1. Phospholipid content (%); 2. cholesterol content(%); 3. amount of cholesterol in wet prawn meat (mg/g).

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